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NEUROPSYCHOPHARMACOLOGY REVIEWS

Biotyping in psychosis: using multiple computational approaches with one data set

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Focusing on biomarker identification and using biomarkers individually or in clusters to define biological subgroups in psychiatry requires a re-orientation from behavioral phenomenology to quantifying brain features, requiring big data approaches for data integration. Much still needs to be accomplished, not only to refine but also to build support for the application and customization of such an analytical phenotypic approach. In this review, we present some of what Bipolar-Schizophrenia Network for Intermediate Phenotypes (B-SNIP) has learned so far to guide future applications of multivariate phenotyping and their analyses to understanding psychosis. This paper describes several B-SNIP projects that use phenotype data and big data computations to generate novel outcomes and glimpse what phenotypes contribute to disease understanding and, with aspiration, to treatment. The source of the phenotypes varies from genetic data, structural neuroanatomic localization, immune markers, brain physiology, and cognition. We aim to see guiding principles emerge and areas of commonality revealed. And, we will need to demonstrate not only data stability but also the usefulness of biomarker information for subgroup identification enhancing target identification and treatment development.

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INTRODUCTION

The Bipolar-Schizophrenia Network for Intermediate Phenotypes (B-SNIP) is a consortium of PIs and associate investigators who focus on understanding the biology of psychosis using phenotypic data from a single large data set. B-SNIP originally formed to seek biomarkers for conventional psychosis diagnoses, so that clinicians could give conventional psychosis diagnoses to individuals more easily, precisely, and consistently. B-SNIP organized its phenotyping across the dimension of psychosis and selected clinical and demographic data as well as candidate biomarkers previously and theoretically informative for psychosis; this included biomarkers derived from cognition, brain imaging, ocular motor recordings, electrophysiology, and genetics, each in addition to in-depth clinical assessment (Table 1) [1]. This initial goal was unfulfilled when it became apparent that no biomarker feature or set of features identified any of the conventional diagnoses, using nearly a thousand individuals, with sufficient statistical power. The conventional DSM-derived diagnoses that we used, schizophrenia (SZ), schizoaffective disorder (SAD), and bipolar disorder with psychosis (BDP), have been established over many years, driven by a need to understand the unexpected clinical phenomena characterizing psychosis, and to communicate about the conditions and manage practical aspects of clinical care. The performance of these categories for leading us to common biological entities, neural mechanisms, or genetic insights has always lacked specificity according to many scientists, who have astutely pointed out that our diagnostic categories are not likely to define pathophysiological entities [2].

Having failed to identify biomarkers to support conventional psychosis diagnoses, B-SNIP set another course: using the biomarker data (Table 1) to identify groups with common neurobiological characteristics, thus clustering psychosis individuals with similar biological profiles [3]. To achieve this, the psychosis cases were pooled (data from 711 probands, 883 relatives, and 278 controls; reserving two biomarkers as external validators), then examined with principal component analysis to reduce the biomarkers to independent "bio-factors". This was followed by *k*-means clustering, using nine distinct "bio-factors" to define the most biologically homogenous clusters of psychosis cases. This strategy generated what we called psychosis Biotypes (Fig. 1). There were several factors essential to this process: (i) large numbers of psychosis individuals to ensure we captured biomarker variance across psychosis; (ii) each individual having large numbers of biomarkers; (iii) extensive quantification within and across biomarker paradigms; and (iv) cutting-edge computational approaches supporting numerical taxonomy. Each Biotype

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Table 1. B-SNIP assessments and tests.

Clinical assessments
Demographic data
Psychiatric, medical, and family history
Structured clinical interview for DSM-IV
Medication, current, and history
Hollingshead SE scale
PANSS; MADRS; YMRS
Lifetime dimension of psychosis scale (LDPS)
Childhood trauma questionnaire (CTQ)
Birchwood SF scale
Akiskal and Barret self-report
Cognition
WRAT-IV
Brief assessment of cognition in schizophrenia (BACS)
Spatial span
PCET; emotion recognition; CPT; SST (computerized)
Neurophysiology
Eye tracking
SPEM
Prosaccade
Antisaccade Tests
Electrophysiology
Resting-state EEG
Auditory paired stimuli ERP
Auditory odd ball ERP
Brain imaging (magnetic resonance)
Structural MR
Resting-state fMRI
Diffusion tensor
MRS

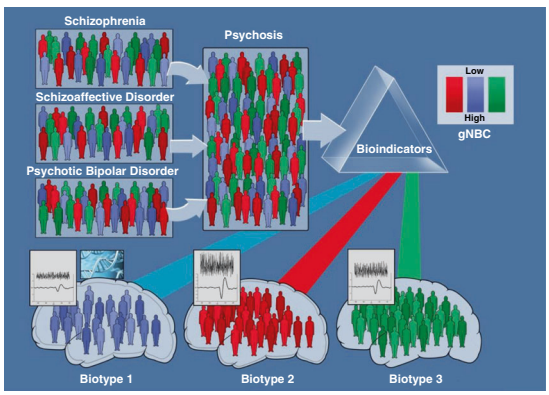

Fig. 1 Bipolar-Schizophrenia Network for Intermediate Phenotypes (B-SNIP) cartoon representing the path to psyhosis Biotypes. B-SNIP study creation of Biotypes from individuals with schizophrenia, schizoaffective disorder, and psychotic bipolar disorder diagnoses. Reported in ref. [3].

contained individuals with all three conventional DSM psychosis diagnoses.

Once we defined psychosis Biotypes, other measures, not part of Biotypes creation, were used as external validators to further illustrate distinct and meaningful group characteristics across the Biotypes. The structural brain volume showed that Biotype-1 (the most clinically severe group) had pervasively reduced cortical volume compared with the healthy controls throughout the whole neocortex. Biotype-2, with a better but still severe clinical and cognitive profile, showed regional reductions in cortical volume from healthy over fronto-temporal regions. And Biotype-3, the least affected psychosis group, showed reduced cortical volume restricted to the core limbic system [4]. Figure 2 shows this pattern. In terms of psychosocial function, each of the three Biotype groups (−1, −2, and −3) showed a step-wise decrement from healthy in psychosocial performance; curiously, their family members showed this as well, albeit, within relatives, all within the normal range.

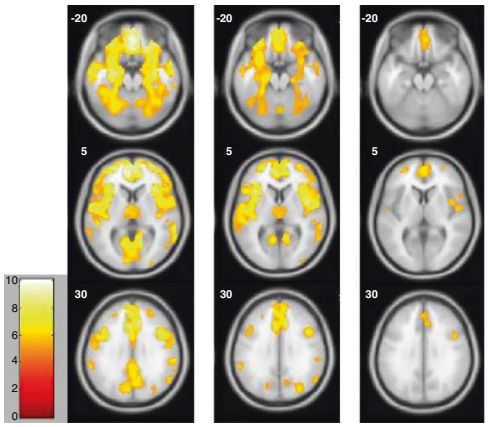

Fig. 2 Average cortical grey matter reduction by Biotype (B-, B-2 and B-3) in the B-SNIP population. B-1 (left), B-2 (middle), and B-3 (right) columns at matched levels show widespread volume reduction from HC in B-1, a substantial reduction in B-2, and localized reductions in B-3; with neocortical distribution in B-1, fronto-temporal, in B-2 and limbic in B-3. Reported in ref. [4].

The next characteristic B-SNIP needed to demonstrate was the replication of Biotypes, all the more compelling because non-replication in the field has become increasingly problematic. B-SNIP2 was born, and B-SNIP1 was repeated five years later, collecting the same biomarkers in similar settings, and increasing the *N* of the psychosis probands in order to make the genetic analyses more informative. We are working on these analyses now. The early indication is that we will successfully replicate psychosis Biotypes (data too preliminary to include). It is a particularly important outcome, considering that B-SNIP2 is an entirely new sample of psychosis and healthy individuals, separated from B-SNIP1 recruitment by 5–9 years. After replication, this biological approach will demonstrate its value when the usefulness of these biomarkers and Biotypes is demonstrated to be etiologically informative and clinically advantageous.

Along this path, many B-SNIP investigator groups mounted "big data" efforts with sophisticated computational methods to examine key features of the data. We describe several of these analyses here to demonstrate the value of applying contemporary computational methods to a common database to develop a unique perspective on one of the field's most important question: what are the neural and biological mechanisms of psychosis?

The task of using biomarker data to understand psychosis, even recognizing the limitations of any kind of brain tests for drawing precise molecular, cellular, and systems conclusions is still daunting because the extent of our knowledge of brain function in psychosis is severely limited. Our mechanistic models remain hypothetical. We are still working without fundamental data, still building up/organizing data from individuals and from animal models of various theories to build psychosis knowledge. We have pulled together a wealth of biomarkers on a large number of individuals within the dimension of psychosis. So, we ask, what

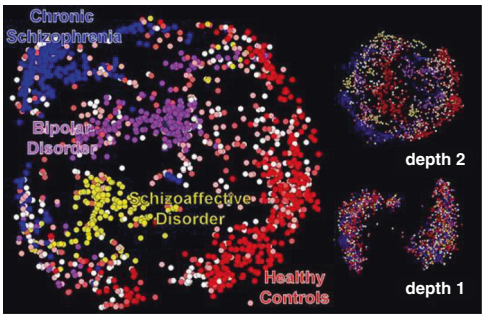

Fig. 3 Big data routines directed to B-SNIP volumetric data. One, two, and three-layer deep belief network trained on structural MRI data from three clinical groups, unaffected relative, and controls. Not previously reported.

can we learn? Which approach may be best? And, how can we arrive at informative biology within the psychoses?

ROLE OF DATA-DRIVEN APPROACHES IN SOLVING "BIG DATA" PROBLEMS IN PSYCHIATRY

Decades of traditional neuroscientific analyses aimed at identifying structural and functional brain differences associated with major psychiatric syndromes have largely relied on univariate statistics and relatively simplistic brain models. These approaches (at least so far) have proved both inadequate in identifying underlying causes of such disorders and in enabling reliable assignment of psychiatric diagnoses on an individual level [5]. Recently, there has been a strong move towards employing multivariate and data-driven approaches that more closely portray complex brain biology [6, 7]. In simplistic terms, big data describes a situation where massive amounts of both structured and unstructured data are collected to solve a problem. Analytic tools are needed to disentangle the complex nature of these data. Instances of this strategy have emerged with the historically large projects such as Enhancing Neuro-Imaging Genetics through Meta-Analysis (ENIGMA), UK Biobank, The Human Connectome Project, The Adolescent Brain Cognitive Development (ABCD) study, and disorder-focused studies such as the Bipolar-Schizophrenia Network for Intermediate Phenotypes (B-SNIP) and the Alzheimer's Disease Research Initiative (ADNI). Combining brain network models with clinical, behavioral, genetic, and cognitive data requires using flexible, data-driven multivariate approaches that acknowledge the complexity of each data type by jointly accounting for their covariance structure [8–10]. Given the inherent density and complexity of neuroimaging data, recent brain network modeling has relied on more sophisticated data-driven techniques such as machine learning approaches, which can either be supervised or unsupervised. Further, we are now solidly in the era of deep learning. This has enabled models that are increasingly flexible, including the ability to capture nonlinear relationships and to identify unknown patterns within a data set that correspond to clinical variables [11]. Deep models can convincingly outperform standard machine learning in a variety of tasks, including classification using brain imaging data [12]. An example of how deep learning can improve our ability to separate clinical groups is shown in Fig. 3. These approaches enable us to visualize the data and identify individuals who are misclassified or lie at the boundary between categories, likely a promising source of information as we work to refine categories or move towards individualized risk markers.

These data-driven techniques are (i) powerful tools to generate and validate hypotheses, (ii) efficient for condensing and reducing large-scale data, (iii) use relatively lenient statistical assumptions,

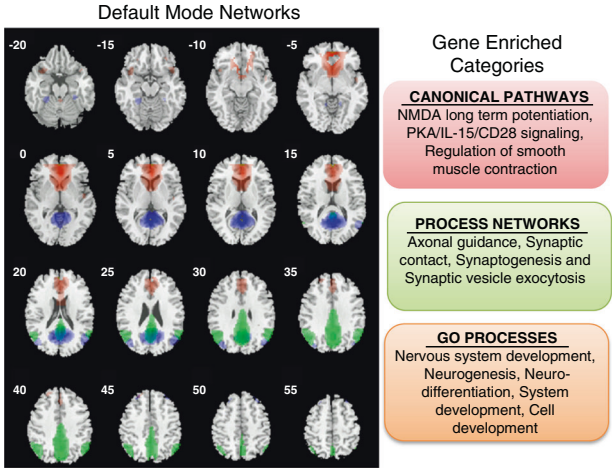

Fig. 4 Gene enrichment in the default mode networks in psychosis. Significant ontology terms derived from a pooled gene enrichment analysis depicting a variety of processes/pathways/networks mediating the risk of psychosis via default mode connectivity. Reported in ref. [13].

(iv) are more effective at partitioning data into signal vs noise, and (v) are able to capture/model brain complexity more accurately. In the B-SNIP project, we used a modified form of ICA called parallel independent component analysis [13], a form of unsupervised machine learning (ML), to automatically cluster and derive links between brain function and genes in psychosis in a bi-multivariate fashion [8, 9, 14] (Fig. 4). This analytic approach serves as an initial step to reduce the phenotypic data in a natural way and to derive relationships between these domains. As an extension, the above-derived metrics or features can then be subjected to the second round of ML clustering that could provide biological clusters across individuals that are segregated or classified based on multimodal biological data.

More recently we applied traditional ML techniques such as support vector machines (SVM) to B-SNIP data to both show and validate the superiority of biologically derived group discrimination (B-SNIP Biotypes) over traditionally segregated groups in terms of brain connectivity in psychosis [15] (Fig. 5). In that study, Regional Homogeneity (ReHo) a metric that measures local brain connectivity, was used as a feature. The same data were analyzed in two ways (a) a traditional ANOVA-based mean difference approach that works at the group level and (b) a multivariate machine learning-based SVM analysis that taps into individualized predictions. Both approaches highlighted the general superiority of clustering data using biological constructs (i.e., B-SNIP Biotypes over traditional phenomenological approaches (i.e., DSM)). However, the fact that SVM-like approaches offer a more in-depth look at individualized predictions is valuable information that is often lost in traditional statistical analyses. For example, using ANOVA we ascertained which specific brain region discriminated groups at the group level. On the other hand, SVM allowed us to ascertain that a multivariate ReHo-based feature set was able to predict whether a given individual belonged to a Biotype class with much higher accuracy and certainty than if they were classified based on DSM stratification. Given that the two methods approach the same data structure using different means, the project was a fruitful exercise to demonstrate the importance of using varied techniques to provide a more complete understanding of brain dysfunction in psychosis. Such efforts using large data sets and multiple data analytic approaches will be critical for generating low-dimensional representations of clinical symptoms, network measures of brain structure/function, and genetics that are useful

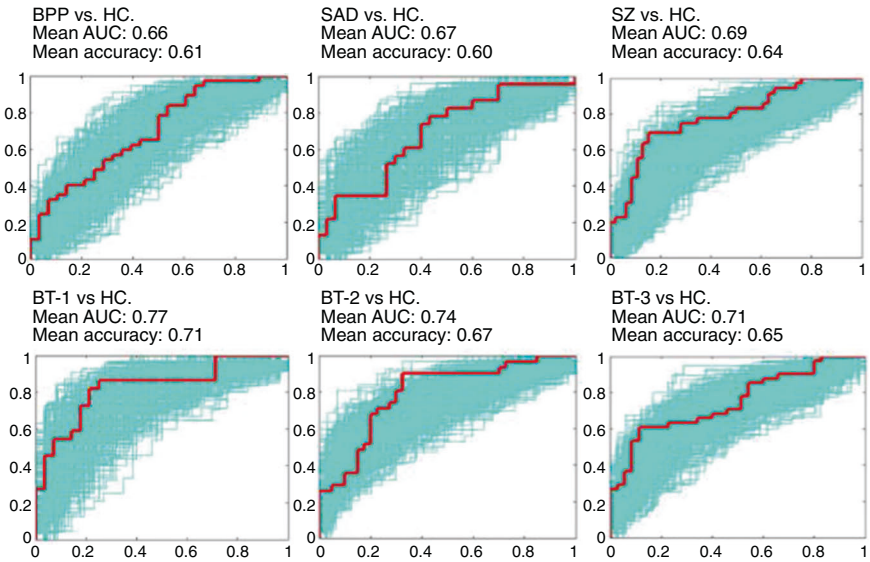

Fig. 5 SVM classification results on regional homogeneity (ReHo) connectivity metrics. Upper: DSM groups with healthy controls (HC); lower: Biotype groups with HC. *X* axis: False-positive rate (specificity). *Y* axis: True-positive rate (sensitivity). Each blue line represents a ROC curve of each SVM with different training-testing separations. Reported in ref. [15].

in the diagnosis and sub-diagnosis of psychiatric syndromes and in paving a path toward future intervention and treatment.

A major challenge is that we have a threefold problem in which the brain, the psychiatric disorders, and the (multimodal) data are all highly complex. Consider the case of clustering, as a general data-driven tool. We can cluster among relatively static variables (such as brain regions or genetic locations) to identify weighted patterns of variables that contributed to a variable of interest. We can also cluster within-subject, across time, for example, to identify transient and more sensitive patterns of functional connectivity [16, 17]. Such dynamic functional brain measures, in our hands, are proving more useful than classic summary measures of resting-state epochs.

Beyond this, we can attempt to jointly identify homogeneous subsets of subjects and data, e.g., using bi- or tri-clustering methods [18]. Perhaps counter-intuitively, this latter approach can enhance our ability to detect differences linked to psychiatric syndromes such as schizophrenia, including the connection to symptoms, by focusing on subsets of subjects who exhibit more homogeneous data. We can also use such approaches to visualize and evaluate individuals on the boundary between groups or evaluate their hierarchical relationship to one another [19]. Finally, if we incorporate continuous measures such as neuropsychological assessments or age, we can identify patterns of data that show graded relations [20]. The use of dimensional measures to assess brain disorders is of particular focus in B-SNIP as well, since we know the boundaries between existing DSM categories are likely not sharp [2]. In summary, our current state of analytic methods allows us to access an unprecedented level of algorithmic flexibility, computational resources, and a growing amount of open data. The challenge is how best to leverage all of these to move the field forward.

DEEP PHENOTYPING PROJECTS, BIG DATA, AND GENOMICS

In psychiatric disorders, studies that define component phenotypes and their genetic associations, offer great promise for illuminating their biological basis. This has led to several large deep phenotyping studies in which many clinical, and brain structure- and function-based phenotypes have been assessed individually in large numbers of genotyped individuals. Deep phenotyping with simultaneous genome-wide analyses serves as a discovery tool for previously unsuspected relationships of phenotypic traits with each other, and with specific molecular involvements. The B-SNIP study includes all of the detailed assessments (Table 1). Genome-wide analyses of such data present challenges, because of the large numbers of phenotypes, daunting (but achievable) sample sizes, appropriate multiple testing corrections for statistical significance, and the resulting computational and statistical burden.

Quantitative neurobiological traits related to brain diseases have become of particular interest since the Research Domain Criteria (RDoCs) initiative [21, 22] which can be seen as expansions of the endophenotype concept, proposed decades earlier by Gottesman [23, 24], similar to Gershon's and Gottesman's 1967 proposal that schizophrenia and its component phenotypes are polygenic. Biological markers, phenotypes, and underlying neurobiological functions related to the disease are conceptualized as continuous "domains" that are more pathological in psychiatrically affected persons. The implicit theory on the genetic architecture of disease is that there are multiple genetic variants with small effects that are correlated with trait markers, and with the right combination the trait markers' quantitative value crosses a threshold for disease liability. The polygenic component of any given endophenotype can be calculated as the additive contribution of many genotypes, including those not significantly associated with the endophenotype or the illness.

An important challenge for analysis of deep phenotyping results is to find a significance threshold based on the family-wise error rate (FWER), (i.e., the probability of Type I error in the entire set of tested hypotheses). An inappropriate statistical significance threshold can mask potential true-positive signals or incur a signal [25]. The probability of a false-positive in at least one of the phenotypes for which GWAS has performed increases with each GWAS (or gene subset analysis). It is tempting to publish component results separately and to restrict multiple test correction to the genotypes in one GWAS. But this gives a false picture of the sample space and adds to the unfortunate number of existing false-positive GWAS results [26].

For the GWAS analysis of any given trait, the correlation structure of phenotypic and genotypic data can be accounted for by determining the "effective number of independent tests" [27].

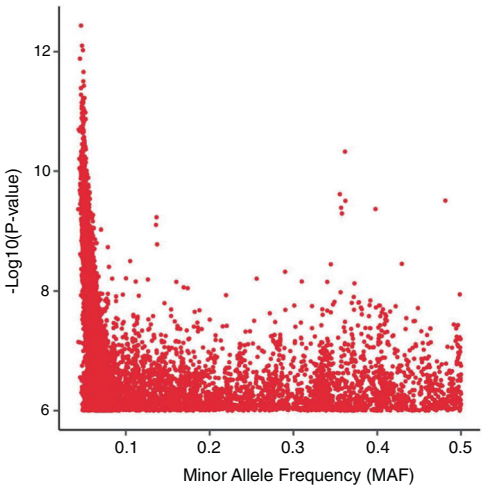

Fig. 6 Effects of permutations on Minor Alle Frequency. Permutation of phenotypes and genotypes gives skewed *p*-values in the low range of common minor allele frequencies. Reported in ref. [30].

M_{eff}-based methods use dimension reduction techniques to filter out the correlation between tests, leaving just the effective number of independent tests, and then apply a Bonferroni correction using M_{eff} instead of the number of genotypes or phenotypes in their respective formulas. As an exercise, we applied M_{eff} dimension reduction methods to 335 individuals with magnetic resonance imaging (MRI) structural phenotypes determined by Freesurfer6 [28]. We did the same with data from B-SNIP [1, 29] for 777 unrelated patients with schizophrenia, schizoaffective disorder, psychotic bipolar disorder, and healthy controls (HC). Using separate M_{eff} estimates for genotypes and for phenotypes, based on various implementations of dimensional reduction of the correlation matrices of each, we obtained FWER-based *p*-value thresholds of roughly 8.4E−13 [30] (Fig. 6). This is considerably more stringent than the uncorrected FWER of 3.5E−11, and the opposite of the reduced stringency we would expect from dimension reduction.

However, there are reports that allele frequency and sample size may affect significance thresholds, and permutation values of the null hypothesis are considered the gold standard for significance thresholds. We shuffled the identification numbers on the genotype data so that the correlations among genotypes and among phenotypes would be preserved. This was a big data problem. Each permutation requires 4.3E6 × 335 tests of association, and these test results must be sorted to find the minimum *p*-value results of all the tests. The minimums for each permutation are then ordered, to estimate the 5th percentile of the minimums as the $p < 0.05$ significance threshold of the FWER. This computation was difficult to perform on the available shared servers in our setting. Nonetheless, the results of the permutations were quite interesting [30]. Although the test results on each GWAS of permuted data followed a uniform distribution as expected, when results were arranged by minor allele frequency (MAF), and we inspected the distribution of the smallest test values, there was an excess of very small *p*-values for MAF < ~0.1. In this data set, then, the FWER threshold for $p < 0.05$, we had observed for the whole data set from permutation (8.4E−13) actually applied only to the lower MAF range for common variants. When we recomputed the statistics for the remainder of the MAFs, the threshold *p*-value was 1.93E−10, which is a bit less stringent than the p-value of the statistical calculations, as expected. Further permutation of these results revealed that this MAF skewing disappears when the permuted data set is ten times larger.

The general conclusion to be derived from this experience with deep phenotype GWAS thresholds is that even though permutation gets cumbersome with the currently huge number of separate tests (because of the large number of random shuffles needed to get reliable genome-wide significance levels) it is less likely to give false positives, which have plagued the GWAS literature [26]. A second conclusion is that we had reached the big data limits of a moderately powerful server.

ANATOMIC FINGERPRINT OF PSYCHOSIS: MULTIDIMENSIONAL ITEM RESPONSE THEORY (MIRT)

The MIRT analysis sought to address the problem of linking neurobiological measures with clinical expression of psychosis [31]. Specifically, the aim was to identify the cortical region(s) in persons with psychosis that are involved in psychotic symptom manifestation [32]. The study asked where in brain greater psychotic symptoms (using PANSS, MADRS, and YMRS) would correlate with the reduced cortical thickness (from MRs processed using FreeSurfer) in the B-SNIP sample using MIRT. We a priori hypothesized that there would exist meaningful anatomic brain regions associated with a psychosis-driven biomarker in this sample. Reductions in cortical thickness have already been widely reported [4]. An anatomic fingerprint could be used to identify regions for further study in psychosis: for example, functionally, with fMRI & electrophysiology and molecularly, with human postmortem brain tissue.

In previous studies that approached this question using total psychosis rating scores for correlations with cortical thinning, the results have been weak [33–35]. Ours is an alternative statistical approach to look at the relationship between psychotic symptoms and regional cortical thickness, using MIRT. The MIRT approach examined both symptomatic (psychosis) and biological (cortical thickness) domains simultaneously while maintaining item-level symptom ratings and regional cortical thickness measures as inputs to the model, simultaneously, aiming to enhance precision in characterizing the relationship [36].

B-SNIP data from 1890 psychosis probands, relatives, and healthy controls (HC), including FreeSurfer (v5.1) analyses, were done as previously described [1]. Characteristics of psychosis were captured with the PANSS, MADRS, and YMRS. The MRI scans on individuals were captured using five different scanners over as many study sites (a GE Signa, a Philips Achieva, a Siemens Allegra, a Siemens Trio, and a GE Signa HDxt) [4]. The analysis in the bifactor model began with 119 variables (68 measures of cortical thickness +51 clinical symptom rating items) with a primary dimension, 9 biological subdomains, and 5 clinical subdomains for a 15 dimension bifactor model. The analysis revealed that 16 of the 68 biological (i.e., cortical thickness) variables loaded strongly (>0.7) on the primary dimension and we used those 16 variables. Moreover, a subset of the 51 clinical variables (i.e., psychosis symptoms) loaded less strongly but still distinctively on the primary dimension (>0.25) to estimate the final bifactor model, that identified the individual clinical variables associated specifically with reduced thickness in the 16 brain regions. In terms of multiple comparisons, the MIRT provides a simultaneous estimation of parameters for all variables in the model and therefore does not require adjustment for multiple comparisons.

The final clinical symptoms which showed >0.25 association with the cortical thickness parameters were: (i) delusions, (ii) hallucinatory behavior, (iii) suspiciousness and persecution, (iv) passive, apathetic social withdrawal, (v) depression, (vi) unusual thought content, (vii) and, active social avoidance. Surprisingly, the set of brain regions where high ratings on the psychosis symptoms (i–vii) associated with the lowest values for cortical thickness all fell onto a large, contiguous brain region, spanning several lobes, creating a “psychosis region”. This region, where

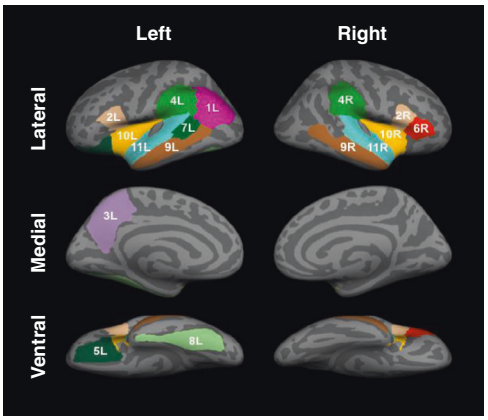

Fig. 7 These regions represent areas where high psychosis symptoms correlate significantly with low cortical thickness. The regions include left (L) and right (R): 1 = inf. parietal ctx; 2 = pars opercularis; 3 = precuneus; 4 = supra-marginal gyrus; 5 = lat. orbitalfrontal C; 6 = pars triangularis; 7 = sup. temporal sulcus; 8 = fusiform gyrus; 9 = mid temporal gyrus; 10 = insula; 11 = sup. temporal gyrus. Reported in ref. [36].

high psychotic symptoms associated with low cortical v0lume, fell directly onto a contiguous swath of neocortex including temporal–parietal–frontal regions (Fig. 7). Curiously, this region includes many of the regions already identified from archival studies as important to psychosis in conventional diagnoses, like the superior temporal gyrus [37] and prefrontal cortex [38].

It is generally assumed that regional cortical thinning is associated with a neuron-sparing pathology in that region. The data here are consistent with the literature in implicating neocortical "thinning" pathology in this area of the neocortex as specifically associated with high clinical psychosis manifestations. This is also consistent with pathology in fronto-temporal connectivity being particularly important to psychosis [38, 39]. The hippocampus is a rather small cortical structure located medial to the involved neocortical regions and is highly connected to many surrounding regions [40]. The hippocampus has often been reported as hyperactive in SZ, especially in early psychosis. The pathology that underlies this hyperactivity has been explored in human tissue [41] and in animal models of the human pathology [42] and a plausible model suggested involving an increase in CA3 excitatory transmission. It could be possible that these alterations within the hippocampus translate into reductions in cortical thickness in nearby tissue. The plausibility of this formulation can be explored in parallel animal models and can be directly examined in human postmortem tissue. As suggested by the MIRT analysis, this is a testable formulation and may generate new knowledge.

DEFICIT SYNDROME ACROSS BIOTYPES

Negative symptoms have received a great deal of attention since they are associated with deficits in functional outcome, poor treatment response, and biological correlates that differ from other symptoms of schizophrenia. Deficit and non-deficit forms of schizophrenia have been proposed based on multiple negative symptoms that are persistent for greater than 1 year and are not fully attributed to symptoms of depression or anxiety, drug effects or environmental deprivation. Deficit and non-deficit forms of schizophrenia differ in several key domains, such as biological correlates, risk factors, and etiology. In B-SNIP, a machine learning approach was taken to redefine psychosis spectrum patients (schizophrenia, schizoaffective disorder, and bipolar disorder with a history of psychosis) into subtypes based on neurobiological

Table 2. Comparison of diagnostic and biotype grouping when stratified by deficit and non-deficit syndrome.

	SZP	SADP	BPP	Fisher's test
Deficit (*n*, %)	153 (45%)	41 (19%)	31 (11%)	$p < 0.001$
Non-deficit (*n*, %)	190 (55%)	171 (81%)	253 (89%)	
	Biotype-1	Biotype-2	Biotype-3	Fisher's test
Deficit (*n*, %)	82 (42%)	60 (26%)	61 (22%)	$p < 0.001$
Non-deficit (*n*, %)	112 (58%)	167 (74%)	213 (78%)	

SZP schizophrenia, *SADP* schizoaffective disorder, *BPP* bipolar disorder with a history of psychosis.

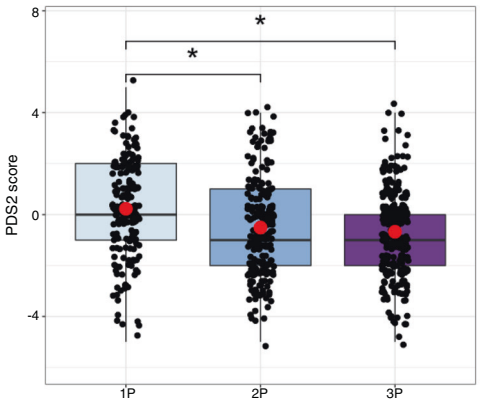

Fig. 8 PDS2 score comparisons across Biotypes. Participants from the B-SNIP1 study underwent deficit syndrome scoring (PDS2) and the boxplot shows contrasts of PDS2 between Biotypes. *$p < 0.001$, (•) mean. Not previously reported.

measures instead of clinical symptoms. This approach identified three biotypes with various degrees of severity from worse to better (BT1, BT2, BT3). Therefore, we proposed that psychosis patients categorized into BT1 were also more likely to have a deficit syndrome compared to the BT3 group.

Utilizing the B-SNIP-1 database, we categorized psychosis probands into deficit and non-deficit groups by subtracting the PANSS negative subscale score for blunted affect by the general subscale score for depression (PDS2) [43, 44]. Participants with a PDS2 score greater than zero were categorized as deficit and those with a score less than or equal to zero were categorized as non-deficit. This resulted in 225 deficit syndrome and 620 non-deficit syndrome groups. In the deficit group there were 153 SZ, 41 SAD, and 31 BPP, while in the non-deficit group there were 190 SZ, 171 SAD, 253 BPP ($chi^2 = 97.9$, $p < 0.001$, Table 2). When stratified by Biotypes the deficit groups consisted of 82 BT1, 60 BT2, 61 BT3, whereas the non-deficit groups consisted of 112 BT1, 167 BT2, 213 BT3 ($chi^2 = 23.2$, $p < 0.001$). BT1 also had significantly higher PDS2 scores compared to BT2 and BT3 ($p < 0.001$), but there was no difference between BT2 and BT3 (Fig. 8). The mean and standard deviation for PDS2 scores were as follows: BT1 (0.23 + 1.87), BT2 (−0.51 + 1.87), BT3 (−0.67 + 1.69). When examining biotype factors by PDS2 scores, it was determined that higher PDS2 scores were associated with poorer biofactor 8 (Eye-tracking saccade task, antisaccade error composite score from principal component analysis) (beta-estimate = 0.079, $p < 0.001$) and biofactor 6 (BACS composite *Z*-score) scores (beta-estimate = −0.139, $p < 0.001$).

Our observations are consistent with previous findings of distinct alterations in white matter microarchitecture [45], prefrontal activation with memory retrieval [46], and neurocognition [47] in deficit vs non-deficit subtypes of schizophrenia. These findings are also concordant with our recent observations of the association between negative symptoms, electrophysiological evidence of diminished neural activity, and cognitive impairments in psychotic disorders [48]. Our findings suggest that such a deficit–non-deficit distinction may cut across psychotic disorders and may provide some support to the biotype-based classification of psychotic disorders.

LINKING PERIPHERAL INFLAMMATION TO BLOOD–BRAIN BARRIER PATHOLOGY

Peripheral markers of dysregulated inflammation and related immunological pathways have received considerable attention in recent years with a strongly established presence in schizophrenia and bipolar disorder [49, 50]. While these findings have been promising, clinical trials examining targeted biologic treatments against IL6 or TNFα have failed to produce clinically meaningful results [51–53]. This lack of connection can be due to several reasons. First, these studies utilized categorical symptomatic constructs for establishing a diagnosis of schizophrenia or bipolar disorder instead of neurobiologically defined categories (e.g., Biotypes). Second, many of the studies examining inflammatory differences between schizophrenia or bipolar disorder patients did not account for the complexity and interactions between peripheral inflammatory markers, as these studies have focused primarily on the unitary effects of inflammatory markers on CNS changes. Third, many of the clinical trials testing the effects of anti-inflammatory treatments in schizophrenia or bipolar disorder have not stratified patients by baseline inflammatory states and/or signatures with the exception for the study by McIntyre et al. [53]. Last, a link between peripheral inflammation and/or signatures of inflammatory markers and their detrimental effects on the CNS are lacking. Therefore, our group approached some of these challenges by examining the evidence for peripheral inflammatory changes comparing neurobiologically defined psychosis groups with traditional diagnostic categories of psychosis, as well as evaluating the potential effects of inflammatory signatures on brain integrity.

Cytokine components of the adaptive immune system, including proinflammatory cytokines (IL2, IFNγ, and TNFα) and anti-inflammatory cytokines (IL4, IL10, and IL13) act as key signaling molecules to coordinate adaptive and innate components of the immune system and exert direct effects on the brain by crossing the blood–brain barrier (BBB) to activate astrocytes and microglia [54]. Cytokines are essential in the development and function of the CNS and are core actors in the maintenance of neuronal integrity, neurogenesis, synaptic remodeling, and neurotransmission [55]. The brain is immunologically privileged due to the BBB limiting cell entry [56]. BBB disruption in psychosis is characterized by increased permeability [57], a greater interface between peripheral and CNS inflammatory signaling [58], and an increase in CNS proteins measurable in plasma [59]. A link between cytokines and the choroid plexus, a contributor to the blood–CSF component of the BBB, has been established in schizophrenia as evidenced by elevations in peripheral cytokines (CRP, TIMP1, MMP9, cortisol, and others) being linked to inflammatory signatures in the choroid plexus [60]. These findings suggest a direct link between the periphery and the upregulation of immune/inflammation-related genes in this key structure. Initial cytokine studies by the B-SNIP group examined 13 analytes in a subset of participants with blood collection for peripheral biomarker studies. We compared levels of individual cytokines and exploratory analyses of cytokine groups across diagnostic and Biotype groups. Two findings consistent with the prior literature

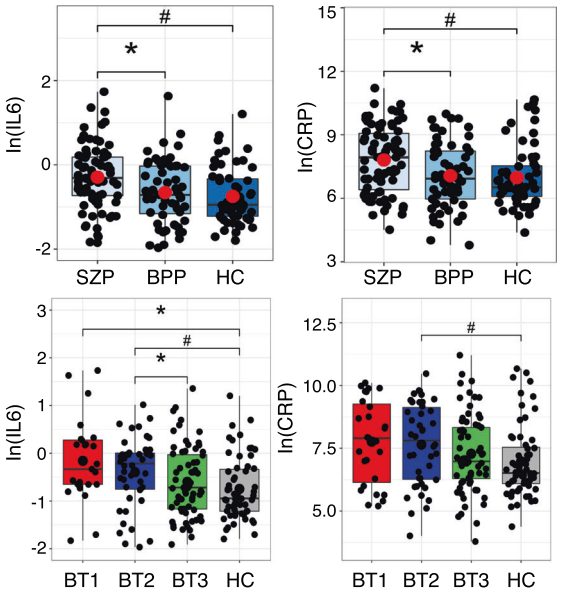

Fig. 9 CRP and IL6 group comparisons. Participants from the B-SNIP1 study underwent analyte level measurements and boxplots showing contrasts of CRP and IL6 between psychosis probands and across biotype groups are illustrated. *$p < 0.05$, **$p < 0.01$, (•) mean. Under review.

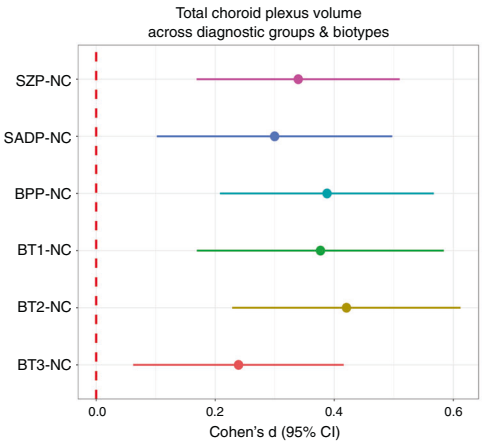

Fig. 10 Choroid plexus group comparisons. Effect sizes for choroid plexus volume comparisons of total choroid plexus volume for schizophrenia (SZP), schizoaffective (SADP), psychotic bipolar I disorder (BPP), and biotypes (BT1, BT2, and BT3) compared to NC. The dot indicates Cohen's *d* and lines indicate confidence intervals (CI). Cohen's *d* estimates were adjusted for age, sex, race, site, and intracranial volume. Under review.

were elevations in IL6 and CRP observed in schizophrenia as compared to controls (Fig. 9). However, when examining these across biotype groups, there were greater differences between groups than in the diagnostic comparisons, particularly notable in IL6 and CRP (Fig. 9). Structural imaging studies of the bilateral choroid plexus volume identified larger volumes in psychosis probands compared with healthy control subjects. It was also noted that there were greater differences when the psychosis probands were stratified by Biotype as opposed to diagnostic groups with the least impairments in BT3 compared to controls (Fig. 10). Increased IL6 and CRP levels were also significantly associated with larger choroid plexus volume in probands. As noted in Fig. 9, CRP and IL6 levels in BT3 were more similar to

controls than the other biotype groups. This finding is of interest because the choroid plexus expresses the receptor for IL6 [61], and in the blood, IL6 has pleiotropic activity, which induces the synthesis of acute-phase proteins (e.g., CRP), activates the acquired immune response by stimulating antibody production and effector T-cell development, and can promote the differentiation or proliferation of non-immune cells [62].

Differences in IL6 and CRP observed across Biotype groups and controls suggested an avenue for further research into the ways in which dysregulation in inflammatory pathways may impact measures of BBB function and pathological correlates. The degree to which IL6 and CRP are selectively related to this, vs a broader multifaceted inflammatory process, is unclear. Exploratory factor analyses (data presented at the poster at the American College of Neuropsychopharmacology in 2019) of a group of 13 cytokines identified 5 notable factor groupings of cytokines. Interestingly, the factor that distinguished BT3 as well as controls from the other BT groups, was comprised of significant loading for not only IL6 and CRP, but also complement 4 (C4a), IL8, IL10, and VEGFD. Examination of diagnoses only noted a separation of schizophrenia participants vs controls. This exemplifies how separate findings from individual cytokines may in fact represent different markers of the same inflammatory process that also includes other influential contributors, and in this example may inform inflammatory mechanisms in part related to altered BBB function.

BASIC NEURO-COGNITIVE CONTINUUM (BANCC)

Biomarkers accounting for the most important initial separation between Biotypes index multiple aspects of cognition (e.g., response inhibition, goal maintenance, response selection, verbal, and working memory). Cognition-related biomarkers also generated the most promising endophenotypes, primarily for Biotype-1 (about a quarter of the B-SNIP sample [3]). Deviation in cognitive functions are traditionally linked to SZ-like psychoses, but they clearly extend to other psychosis syndromes [63–67] as well as to syndromes with genetic and phenotypic overlap with the psychoses [68–70]. In addition, cognitive ability, when left untreated, maybe the best predictor of course and functional outcomes across the psychoses [71, 72].

Cognitive ability, of course, is a manifestation of the structural and functional integrity of the brain. This fact has fueled the logical extension of cognitive ability research in psychosis to related neuroimaging domains. McTeague et al. [68, 73] draw the reasonable conclusion that there is a transdiagnostic latent feature accounting for a broad vulnerability to serious psychiatric syndromes, and this latent feature is indexed by common and broad neurobiological measures of cognition, brain structure, and neural function. Understanding the ubiquitous nature of these inter-related neurobiological features may offer an opportunity for appreciating a dimensional vulnerability for serious psychopathology that does not respect conventional clinical diagnostic boundaries [67, 68, 74, 75].

McTeague et al. [73] predicted that research addressing the relevance of a multi-trait neurocognition continuum must be both transdiagnostic and dense in phenotype measurement. Although B-SNIP does not have sufficient data on syndromes other than idiopathic psychoses, we do have extensive phenotyping data at multiple levels of analysis [1, 29, 76]. As such, we were able to evaluate the extensiveness of phenotypic overlap between a multimodal cognition construct and multiple neurobiological features for individual subjects across the psychosis dimension.

Across B-SNIP1 psychosis cases (n-711), their relatives ($n = 883$), and healthy persons ($n = 278$), we evaluated relationships between a multivariate dimension of cognitive ability (what we called "Cognitive control") [3] and multiple sets of measures not used to construct this dimension. These measures included: (i) structural MRI (397 Freesurfer variables characterizing cortical volume, surface area, and thickness across multiple regions), and (ii) cognition-psychophysiology measurements (20 neuropsychological, EEG/ERP, and ocular motor measures). Within each measurement set (397 structural MRI measures; 20 cognition-psychophysiology measures) we computed the subject–level relationships between all individual measures simultaneously and the multivariate dimension of cognitive ability [77]. Random effects were the intercepts and predictors (e.g., MRI or cognition-psychophysiology). We estimated individual variable-specific associations in relation to the overall measurement set association using empirical Bayes estimates. This analysis determined if each individual measure in a set (MRI or cognition-psychophysiology) fit a single function on cognition, so the purpose of this analysis was to discover how the overall association varied across measures within a set.

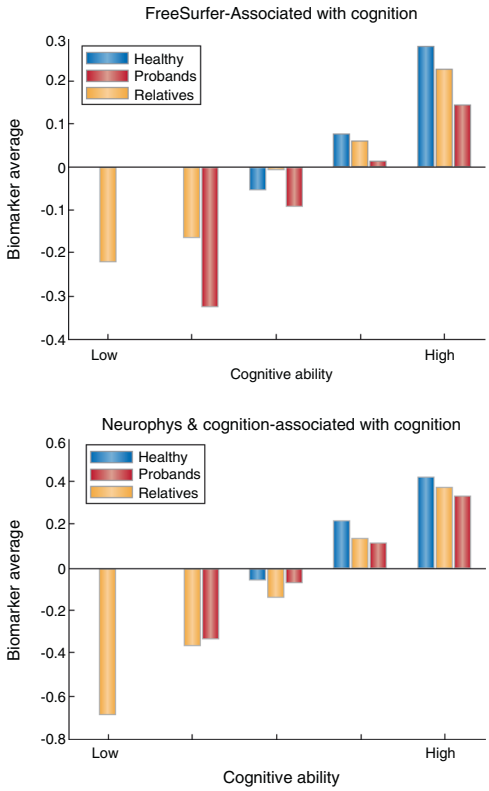

Fig. 11 Overall structural MRI (top plot) and cognition and neurophysiology (bottom plot) standard scores as a function of cognitive ability. The cognitive ability dimension is plotting in quintiles by different subject groups (healthy in blue, probands in orange, relatives of probands in yellow). When considering all measures in a set, all groups show declines in both MRI and neurophysiology and cognition from high to low cognitive ability levels. Not previously reported.

Figure 11 shows bar plots averaging over variables within a measurement set for MRI and cognitive-psychophysiology; other sets have the same pattern. These plots show mean values within cognitive ability quintiles (x axis) for probands, relatives, and healthy subjects. Note that probands span the cognitive ability range but the overwhelming majority of broadly recruited healthy subjects occupy the upper 3 quintiles (no blue bars in lower 2 quintiles). The functions (slopes and y-intercepts) are statistically equivalent for probands, relatives, and healthy subjects. The overwhelming majority of measures are captured by the overall functions in Fig. 11: (i) structural MRI (slope on cognition = 0.22 in standard units, SE = 0.0052, $p < 0.0001$)—379 of 397 (95.5%) fit this function; (ii) cognition-psychophysiology (slope = 0.43 in

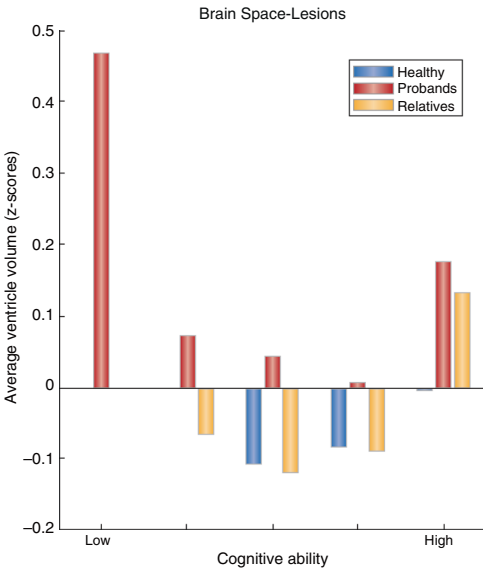

Fig. 12 MRI measures that deviate from the BANCC pattern. Brain space volumes are plotted by cognitive ability (in quintiles) and subject group (healthy in blue, probands in orange, relatives of probands in yellow). Brain space measures, like ventricular volume, are a set of variables that do not follow the overall pattern displayed in Fig. 11, with especially probands showing a marked deviation from the canonical pattern at the deficit end of cognitive ability. Not previously reported.

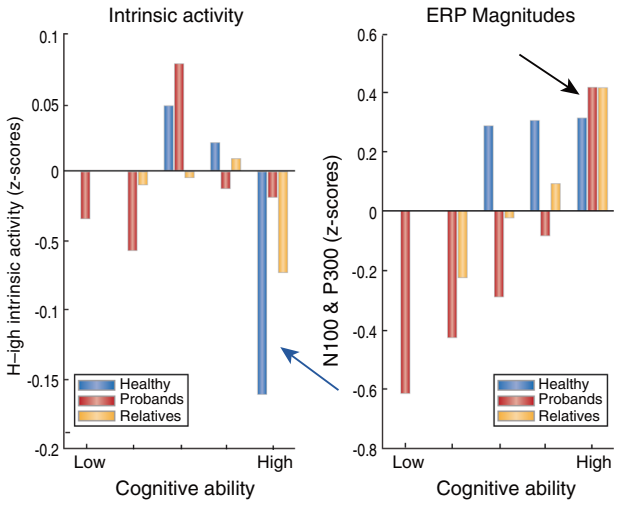

Fig. 13 EEG measures that deviate from the BANCC pattern. Intrinsic EEG activity (left plot) and ERP magnitudes like N100 and P300 (right plot) are displayed as a function of cognitive ability quintiles and subject group (healthy in blue, probands in orange, relatives of probands in yellow). The intrinsic activity does not follow the canonical pattern of Fig. 11 for any group; ERP magnitude does not follow this pattern for healthy persons, meaning they are able to generate ample ERP responses independent of cognitive ability, giving them an advantage in signal-to-noise ratio over a broader range of cognitive abilities. At the high end of cognitive ability, healthy persons have a decided signal-to-noise advantage given their very low intrinsic activities (blue arrow) in combination with robust neural responses to salient stimuli (black arrow). Not previously reported.

standard units, SE = 0.0088, $p < 0.0001$)—13 of 20 fit this function (65%). These analyses indicate the extensiveness of the transdiagnostic neurocognition construct described by McTeague and others [69, 70, 73, 74, 78]. We call the overall pattern of associations across these hundreds of laboratory measures the BAsic Neuro-Cognitive Continuum (BANCC). BANCC has a deficit and surplus extremes and may index the multifactorial background that accounts for similarities between syndromes. BANCC quantifies cognitive dysfunction and associated biomarkers. An important question is whether a psychosis person's BANCC location is immutable or can be changed with targeted intervention, along with concomitant changes in associated features (like functioning and symptomatology).

The overwhelming number of structural MRI and cognition-psychophysiology variables that fit the BANCC, at first blush, may yield the impression there is no other meaningful variance to explain. There are counterparts to BANCC, however, that we believe are indexed by variables not captured by this primary dimension. One possibility is that measures orthogonal to BANCC identify phenotypic expressions of distinct diseases, or "bio-indicators", of specific psychosis pathophysiology. These bio-indicators are superimposed on BANCC. We present four illustrations of this possibility and its theoretical relevance for parsing specific etiologies from the multivariate-polygenic background of BANCC.

First, for structural MRI there were two complimentary findings. Variables statistically deviating from BANCC included (i) almost all of the hippocampal measures, and (ii) gross measures of brain space enlargements and/or lesions (e.g., all ventricular volumes, white matter signal abnormalities, overall CSF). The entire hippocampal complex bilaterally showed a more devastating decline over cognitive ability than all other brain measures (they have steeper slopes than evident for the canonical pattern of Fig. 11). The hippocampal complex is central to neurodevelopmental models of psychosis (31). All the brain space-lesion measures had the same distributional properties; their average is displayed in Fig. 12, illustrating a discontinuity within probands at the deficit cognitive ability end; (iii) in our genetics data the temporal horns of the lateral ventricles [79, 80] have a specific genome-wide significant hit within Neurexin 1 (NRXN1), important in synapse formation and whose rare complete deletion is associated with psychosis and autism [81]. These findings indicate the hippocampal complex and adjacent structural alterations may be critical for indexing the specific etiology of a psychosis subgroup [32, 41].

Second, for the psychophysiology set, there were two additional complimentary findings: (i) measures of intrinsic brain activity, which specifically indexes Biotype-2, statistically deviated from BANCC. Their distributional properties are displayed in the left half of Fig. 13; and (ii) ERP magnitudes to salient stimuli, which specifically indexes Biotype-1, track with BANCC for probands and relatives but not for healthy people (see right half of Fig. 13). At the surplus end of cognitive ability, all groups have statistically similar ERP magnitudes (black arrow), but healthy subjects have considerably lower intrinsic activity (red arrow). Healthy persons, but not probands and relatives, maintain ERP magnitudes as cognitive ability declines. Signal fidelity is always a function of (i) background neural activity against which the signal-specific neural response is generated, and (ii) signal strength to salient stimuli. Even when psychosis cases generate a robust neural response to salient stimuli, their signal-to-noise ratios (ERP magnitude/intrinsic activity) are low. Signal-to-noise becomes especially dire in psychosis as cognitive ability declines because background noise is elevated and signals related to information processing are diminished.

The above presentation supports propositions [73] that (i) multimodal assessments are necessary to appreciate both the ubiquity of BANCC regardless of psychiatric status [75, 82], and (ii) that there may be distinct deficits not captured by this construct. Our analyses indicate that despite the overwhelming phenotypic variance captured by BANCC, there are some biomarkers that

deviate from the canonical pattern (what we propose as bio-indicators) that may more closely index diseases within psychiatry, at least within what is now idiopathic psychosis.

There are two additional possibilities stimulated by these analyses that may be usefully considered in subsequent investigations. First, it is often assumed that working within a level of analysis, and finding of shared variance patterns within, for instance, diverse signs/symptoms [83] or multiple cognitive abilities, will support identifying associations across levels of analysis (e.g., describing how psychosis symptoms map to neurobiology or how cognition relates to brain structure). Data from B-SNIP indicates these approaches yield strikingly different outcomes. For instance, shared variance in psychosis-related symptoms do not map to the same symptom domains as when symptom variance is parsed in relation to neurobiology [84]. Likewise, when describing shared variance within cognition alone, a common "cognitive ability" dimension captures the overwhelming share of variance across diverse cognitive tests [85]. But when extracting shared variance between cognition and brain structure [86] or cognition and diverse saccade tasks (Huang et al., under review), specific cognitive abilities are extracted in relation to the anatomic and ocular motor performance that is different from the generalized "cognitive ability" dimension. Which approach provides the most veridical outcome in different applications is an empirical question.

Second, numerous investigators propose that serious psychopathology is a dimensional extension of normalcy at the extreme end of a multifactorial liability continuum. This appears to be true for a primary dimension like BANCC. But it is also possible that bio-indicators index specific etiologies in a discrete rather than continuous fashion that dictate manifestation of symptomatic pictures we equate with psychosis syndromes. One possibility is that an individual, based on constitutional factors and environmental circumstances, may fall anywhere along BANCC, but an individual would only manifest a psychosis syndrome in the presence of one or more psychosis-relevant bio-indicators. Whether these additional discrete "hits" have specific effects or move an individual toward the deficit end of BANCC may be specific to different bio-indicators as they increase the likelihood of the development of a more serious version of any psychiatric disease [70]. This is different from models assuming psychosis risk is continuously distributed in the population [23, 78, 87].

FUTURE RESEARCH DIRECTIONS

Soon, B-SNIP will finish the replication analysis, to test the extent of the replicability of brain-informative biomarkers in individuals with psychosis and healthy volunteers. If replicable, it will confirm the potential usefulness of these kinds of biomarkers over time for disease characterization and whether or not and how these measures need to be adjusted for future discovery. Nonetheless, even if replicable, the biomarkers and psychosis Biotypes will still have to be useful for disease understanding or treatment guidance. Biomarkers to mark disease constructs would be important; biomarkers to guide treatment decisions would be clinically useful; biomarkers to guide drug discovery would be helpful. Some pathological biomarkers have already suggested potential pathways to novel treatments or selective drug responses, which are being pursued. We are pursuing practical ways of applying these B-SNIP outcomes to clinical advantage for individuals with psychosis.

DISCUSSION

These computational vignettes from B-SNIP were conducted to gain an understanding of psychosis biology and to test the disparity between phenomenologically and neurobiologically defined psychosis groups. Exploration of these ambitions has only begun. With key phenotypes, especially the phenotype bio-factors, neurobiologically defined psychosis groups like the psychosis Biotypes showed distinctive characteristics, suggesting the more homogeneous underlying biology hypothesized within Biotypes. We learned preparatory lessons about working with biologically similar groups; and we gained an appreciation for the remaining heterogeneity within even biomarker-defined psychosis groups, like Biotypes. The cross-dimensional characteristics measured in B-SNIP include those which are highly robust (genetic measures, brain structure, electrophysiology) as well as those less robust (clinical assessment, symptom complexes, psychosocial function). All of the biomarkers across all levels of function need to be assessed for secondary influences like alertness, sex, race, circadian influences, and medication effects. More will be done as these studies move forward. Performing computations across the levels of analysis were key approaches that often identified Biotype differences and distinctions. We believe that, while phenomenology is critical for describing many aspects of psychosis course, prediction, and care, the study of neurobiological-defined groups will advantage cellular, molecular, systems, and target discovery, which is the goal in our B-SNIP studies.

In this paper, (i) we initially reviewed the B-SNIP use of a comprehensive biomarker battery to define bio-factors, then Biotypes for psychosis; then we added vignettes from projects that test specific questions both trans-diagnostically and within the Biotypes, often using big data approaches and frequently across multiple levels of analysis. Early on, (ii) B-SNIP piloted the application of cutting-edge computational approaches for large data sets to identify maximally informative methods. (iii) We have looked at genetic associations for biomarkers and Biotypes and noted that the statistical rigor designed into genetic analyses may be more demanding than needed for biomarker-gene associations; this sets a new target for future genetic studies that transcend the use of clinical definitions alone. When we wanted to show a relationship between a clinically important measure (i.e., psychosis) and a neurobiologically meaningful outcome (i.e., cortical thickness) we turned to MIRT to query the anatomy of psychosis. The answer from MIRT analysis demonstrated a broad and contiguous expanse of neocortex covering parietal–temporal–frontal areas, which is associated with psychosis manifestations. It is up to us to follow this finding up, perhaps first with brain imaging, then, in human postmortem tissue studies, even though the anatomic target remains broadly defined. (iv) Looking again at clinical symptoms and the Biotypes, we show that the B-1 Biotype has more individuals with high negative symptoms than the other Biotypes, suggesting an approach to enrich a population for specialized treatment testing. Also, (v) since B-SNIP appears to be an ideal group in which to examine immune factors in psychosis—given its high volunteer numbers, its subject diversity, and adequate blood samples—we performed an initial analysis of immune factors in conventional diagnoses and in Biotypes. We report, not a single immune factor, but a complex "fingerprint" of immunologically significant factors is most discriminating. These numbers will be greatly enhanced with the B-SNIP2 population and results will be more definitive at that point. These results will contribute to current conversations of blood immune factors in psychosis pathophysiology. (vi) This paper ends with the observations of a large cognition-driven continuum in individuals across all B-SNIP volunteers, showing that cognition correlates with many brain biomarkers across all biomarker families. Therefore, to advantage disease understanding, these continuous cognition-associated data call attention to the biomarkers that fall outside of the continuum as targets. We have identified several of these non-continuum biomarkers (possibly, bio-indicators of disease) and will use them in future studies to identify target engagement for

novel treatments. It is a rational approach to treatment discovery in psychosis.

It seems probable from initial B-SNIP studies that we will gain pathophysiological understanding gradually, slowly, and episodically. These studies show that large case numbers are essential, that computational approaches designed not only for a large data set but for analyses of complex targets like the brain are needed. Moreover, the use of multiple biomarkers representing different levels of function is necessary. We may have expectations for fast and efficient answers, where the critical clinical need is constantly pushing our research. In the area of psychosis and serious psychiatric illness, the magnitude of the need and of individual personal suffering drives our work every day. We may benefit from some auspicious observations and fortuitous connections to help us along, as has happened before in our field. Still, in order to identify clear molecular and systems targets, the connections we can make between biomarkers and clinical characteristics need to be molecular and regional, with systems implications. For us, every discovery and advance in neural understanding provides another step to use in clinical discovery. We know already that we will need to be able to move from biomarkers to the molecular level and to an understanding of their regional neuroanatomical and functional implications in the context of the brain's tremendous complexity. This includes what we still need to learn about the connections between several levels of data, as we find informative levels of analysis. It is a task we have dedicated our efforts toward in the previous sections, and note that such efforts do not yet yield a clear unified picture. Complexity remains for ongoing and future efforts to address these issues, yet new directions are being generated through "big data" methods, and large amounts of variance from a range of phenotypes are being successfully sorted into meaningful constructs.

We currently ask many questions of our biomarkers, including how to make in vivo biomarkers molecularly informative. To advantage this, we can test functions pharmacologically. We can try to use case-specific stem cells in informative designs with molecular readouts. We can attempt to bring biomarker information to the level of genetic markers, as our numbers grow to link these two areas: phenotypic and genetic data. And we can use complex biomarker "fingerprints", if you will, to expand the information content of the biomarker tools.

There are approaches to link human biomarkers with parallel readouts in animals, where molecular and cellular pathology can be manipulated. This could provide clear molecular hypotheses to explain alterations in human biomarkers, making the biology of the biomarker apparent. This will allow us not only to use a biomarker as just that—a marker to code a biological phenomena—but also as an indication of the pathobiology of that change. We hope to have as clear biomarkers for our brain disorders as we have clear measures for other disease functions, like blood pressure, EEG, and chest fluoroscopy. Even with tools like these, the treatment of the psychiatrically ill will be difficult because of the extensive complexity of the brain as a system. To attain success, we will have to embrace the complexity of the brain, recognizing the brains' plasticity, its systems, its individual distinctiveness, and all of the gender and racial diversity we know is common to brain function.

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AUTHOR CONTRIBUTIONS

CT takes overall responsibility for the paper and composed the Introduction and Discussion. Specific sections were composed by GP, SAM, VDC; ESG, NA-R, SK, HA; CT, RG, EII, MH-H; MK; JRB, PL; BAC, JM in order. All authors commented and edited the whole paper extensively.

ADDITIONAL INFORMATION

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